
**Faculty of Resource Science and Technology**

# Chitosan Derived Nanoparticles –based Sensing Probes for Detection of Japanese Encephalitis (JE) Antigen

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# Chitosan Derived Nanoparticles – based Sensing Probes for Detection of Japanese Encephalitis (JE) Antigen

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## DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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## ABSTRACT

Japanese encephalitis virus (JEV), a mosquito-transmitted flavivirus that will lead to neurological diseases and death in humans in extreme cases. Up to date, there are currently no specific anti-viral medications and curative treatment for JEV. Therefore, early, effective and accurate diagnosis of JEV infection by using a point-of-care, simple, rapid and economical biosensor is very critical for the prevention and control of JEV outbreaks. A biosensor is an analytical device containing biocatalyst and a transducer for point-of-care screening which converts any diseases or harmful biological events to detectable signals. Designing and fabricating biosensors with a higher rate of reaction and sensitivity has to become popular among researchers due to the emergence of nanotechnology. In recent decades, carbon nanoparticles (CNPs) have attracted numerous researchers to study their nanoscience and nanotechnology potential comparing to other nanoparticles due to their special characteristics especially their optical and electrochemical properties which turn them into potential candidates for bioimaging, biosensing, drug delivery, and photodynamic applications and most importantly CNPs are easier to synthesize, environmental friendly and low cost. In this study, based on the electrical conductivity performance of CNPs derived from the various types of precursor characterized by using cyclic voltammetry, (CV) and electrochemical impedance spectroscopy, (EIS), chitosan CNPs with the smallest size and performed highest electrical conductivity are selected for further modification in order to fabricate a higher rate of reaction and sensitivity of electrochemical biosensor. Carbon nanoparticles modified on screen-printed carbon electrode (CNPs-SPCE) electrochemical biosensor strip has been successfully fabricated by immobilized JEV antibody onto the surfaces of carbon nanoparticles through amide bonds formed between amino groups of CNPs and carboxylic groups of JEV antibody. The analytical performance of the CNPs-

SPCE electrochemical biosensor strip was characterized using CV and EIS. CNPs-SPCE electrochemical biosensor strip exhibited a linear detection range of 1 – 20 $ngmL^{-1}$ with a low limiyt of detection (LOD) of 0.36 $ngmL^{-1}$ (at S/N = 3) for JEV, detection sensitivity was 0.024 $ngmL^{-1}$ for JEV and analysis results were obtainable within 10 minutes. In order to enhance the sensitivity and selectivity of the CNPs-SPCE electrochemical biosensor strip, the addition of Gold nanoparticles (AuNPs) onto CNPs to produce the hybrid Gold-Carbon nanoparticles (Au-CNPs) which provides a higher effective surface area, catalytic activity and electrical conductivity has been successfully fabricated. The analytical performance of hybrid Gold-Carbon nanoparticles modified on screen-printed carbon electrode (Au-CNPs-SPCE) electrochemical biosensor strips was characterized using CV and EIS. Au-CNPs-SPCE electrochemical biosensor strip exhibited a linear detection range of 1 – 20 $ngmL^{-1}$ with an extremely low LOD of 0.29 $ngmL^{-1}$ (at S/N = 3) for JEV, detection sensitivity was 0.04 $ngmL^{-1}$ for JEV and responding time is 10 minutes. The potential clinical application of this Au-CNPs-SPCE electrochemical biosensor strip was demonstrated by the detection of JEV in human serum while the selectivity of this biosensor strip was also proven by using Dengue antigen. In conclusion, virus infections can cause serious diseases to humans and animals as certain viruses could spread rapidly within a very short period. Hence, the fabrication of this accurate and fast biosensor for early detection of viruses is often crucial for clinical diagnosis and therapy.

**Keywords:** Carbon nanoparticles (CNPs), Japanese encephalitis virus (JEV), electrochemical biosensors, hybrid gold-carbon nanoparticles (Au-CNPs), chitosan

***Alat Pengesan Sensor Berasaskan Nano-Zarah Karbon Diperolehi dari Kitosan untuk Mengesan Antigen Japanese Encephalitis (JE)***

## *ABSTRAK*

*Virus Japanese encephalitis adalah sejenis flavivirus yang berjangkit melalui gigitan nyamuk boleh membawa kepada penyakit neurologi dan juga kematian jikalau jangkitan itu menjadi semakin teruk. Sehingga kini, tiada ubat anti-virus dan rawatan khas untuk JEV. Oleh itu, diagnosis yang lebih awal, cepat dan tepat bagi jangkitan JEV adalah sangat penting untuk mencegah dan mengawal wabak JEV merebak. Biosensor adalah peranti analisis yang mengandungi bio-mangkin dan transduser yang boleh mengesan apa-apa penyakit atau aktiviti biologi yang berbahaya dan mengubah kepada isyarat yang dapat dikenalpasti. Reka bentuk dan fabrikasi biosensor dengan kadar tindak balas dan sensitiviti yang tinggi adalah sangat popular di kalangan para penyelidik sejak kemunculan bidang nanoteknologi. Kebelakangan ini, CNPs telah menarik perhatian ramai penyelidik untuk mengkaji potensi dan keberkesanan kegunaannya dalam bidang nano-sains dan nano-teknologi berbanding dengan nano-zarah yang lain. Ini adalah kerana ciri khusus mereka terutamanya sifat optik dan sifat elektrokimia yang menjadikan CNPs sebagai calon pilihan utama untuk bioimaging, biosensing dan yang paling penting adalah CNPs mesra alam, proses penyediaan yang lebih mudah dan murah. Dalam kajian ini, berdasarkan kepada prestasi kekonduksian elektrik untuk pelbagai jenis CNPs yang diuji dengan menggunakan CV dan EIS, kitosan CNPs dengan saiz terkecil dan kekonduksian elektrik tertinggi dipilih untuk kajian selanjutnya supaya untuk menghasilkan biosensor yang mempunyai kadar tindak balas dan sensitiviti yang tinggi. Fabrikasi biosensor elektrokimia CNPs-SPCE berjaya disediakan dengan pembentukan ikatan amida antara kumpulan berfungsi -$NH_2$ dari CNPs dan kumpulan berfungsi COOH dari antibodi JEV. Prestasi biosensor*

*elektrokimia CNPs-SPCE diuji melalui CV dan EIS. Strip biosensor elektrokimia CNPs-SPCE mempamerkan julat pengesanan linear antara 1 hingga 20 ngmL$^{-1}$dan mempunyai LOD yang rendah iaitu 0.36 ngmL$^{-1}$ (pada S / N = 3) , kepekaan pengesanan untuk JEV ialah 0.024 ngmL$^{-1}$ dan keputusan analisis boleh didapati dalam masa 10 minit. Untuk meningkatkan kepekaan dan kepelbagaian biosensor elektrokimia CNPs-SPCE, penambahan AuNPs keatas CNPs untuk menghasilkan hibrid Emas-Karbon nano-zarzh (Au-CNPs) yang mempunyai jumlah luas permukaan yang lebih tinggi, aktiviti mangkin, kekonduksian elektrik dan bio-keserasian yang jauh lebih berkesan. Prestasi biosensor elektrokimia Au-CNPs-SPCE mempamerkan julat pengesanan linear 1 hingga 20 ngmL$^{-1}$ dengan LOD yang sangat rendah iaitu 0.29 ngmL$^{-1}$ (pada S / N = 3) kepekaan pengesanan ialah 0.04 ngmL$^{-1}$ untuk JEV dan masa yang bertindak balas adalah 10 minit. Potensi penggunaan klinikal untuk biosensor elektrokimia Au-CNPs-SPCE ini ditunjukkan oleh pengesanan JEV dalam serum manusia dan kajian pemilihan virus turut dibuktikan dengan menggunakan antigen denggi. Kesimpulannya, jangkitan virus boleh membawa penyakit yang serius kepada manusia dan haiwan disebabkan terdapat sebahagian virus tersebut boleh menyebar dengan cepat dalam tempoh yang sangat singkat. Oleh itu, pengesanan jangkitan virus dengan lebih awal, berkesan dan cepat adalah sangat penting untuk diagnosis dan rawatan di klinik.*

***Kata kunci:*** *Nano-zarah karbon (CNPs), virus Japanese encephalitis (JEV), biosensor elektrokimia, hibrid emas-karbon nano-zarah (Au-CNPs), kitosan*

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| | |
|---|---|
| Au-CNPs | Hybrid Gold-Carbon nanoparticles |
| Au10-CNPs | Hybrid 10 nm Gold-Carbon nanoparticles |
| Au20-CNPs | Hybrid 20 nm Gold-Carbon nanoparticles |
| Au40-CNPs | Hybrid 40nm Gold-Carbon nanoparticles |
| Au-CNPs-SPCE | Hybrid Gold-Carbon nanoparticles modified on screen-printed carbon electrode |
| BSA | Bovine serum albumin |
| CNs | Carbon nanomaterials |
| CNTs | Carbon nanotubes |
| CNPs | Carbon nanoparticles |
| CNPs-SPCE | Carbon nanoparticles modified on screen-printed carbon electrode |
| CV | Cyclic voltammetry |
| DNA | Deoxyribonucleic acid |
| EDC | N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride |
| EIS | Electrochemical Impedance Spectroscopy |
| FTIR | Fourier Transform Infrared Spectroscopy |
| GCE | Glassy carbon electrode |
| GO | Graphene oxide |
| JE | Japanese encephalitis |
| JEV | Japanese encephalitis virus |
| LOD | Limit of detection |
| MAC-DOT | IgM capture dot enzyme immunoassay |

| | |
|---|---|
| MAC-ELISA | IgM antibody capture enzyme-linked immunosorbent assay |
| MW-CNT | Multiple wall carbon nanotube |
| NDs | Nanodiamonds |
| NHS | N-Hydroxysuccinimide |
| PBS | Phosphate buffer saline |
| PRNT | Plaque reduction neutralization test |
| $R_{et}$ | Electron-transfer resistance |
| RNA | Ribonucleic acid |
| RT-PCR | Reverse transcription polymerase chain reaction |
| SAM | Self-assembly method |
| SCE | Standard calomel electrode |
| SEM | Scanning electron microscopy |
| SHE | Standard hydrogen electrode |
| SPCE | Screen-printed carbon electrode |
| SW-CNT | Single wall carbon nanotube |
| UNIMAS | Universiti Malaysia Sarawak |
| UV-Vis | Ultraviolet visible Spectroscopy |
| WHO | World Health Organisation |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background and Problem Statement

Japanese encephalitis virus (JEV), a mosquito-transmitted flavivirus consisting of a positive-sense single-stranded ribonucleic acid (RNA) genome which acts as messenger RNA encoding a single open reading frame (Yun & Lee, 2014). This will then lead to neurological diseases and death in humans in extreme cases (Li et al., 2015; Bharucha et al., 2018). World Health Organisation (WHO) has reported that JEV outbreaks first happened in Japan during the 19th century. In recent years, JEV has become a major public health concern globally as there are estimated 70,000 human cases and 20,000 deaths annually (Nain et al., 2016). Mansfield et al. (2017) pointed out some reproductive problems such as abortion, still-birth and birth defects happening to adults infected by JEV. On the other hand, JE is the major cause of encephalitis and 75% happened to children less than 15 years old in Asia (Mansfield et al., 2017). According to Zanin et al. (2003), vaccination for JEV has been available and used internationally since the 1930s and the commercially used vaccines now in the market are JE-VAX and JE-VC, respectively. However, Mansfield et al. (2017) reported that hypersensitivity and licensed reason in certain countries are some of the reasons for the uncontrollable outbreaks of JEV in endemic countries. Accurate diagnose is therefore important to prevent and control the JEV outbreaks.

Some conventional diagnostic devices have been used for the diagnosis of JEV infection, for example, enzyme-linked immunosorbent assay (ELISAs) method, plaque reduction neutralization test, reverse transcription-polymerase chain reaction (RT-PCR) and virus isolation (Hobson-Peters, 2012). These methods involve costly equipment and

specialized expertise to run the laborious and slow analyses. A simple nitrocellulose membrane-based immunoglobulin M (IgM) capture dot enzyme immunoassay (MAC DOT) was developed and commercialized for the diagnosis of JE (Solomon et al., 1998). The IgM antibody capture (MAC ELISA) is the first-line serological assay recommended by the World Health Organization (WHO) for JE diagnosis. There are several types of MAC ELISA kits currently available commercially. However, studies conducted on three of the most common MAC ELISA kits (Panbio JE-Dengue IgM combo ELISA, XCyton JEV CheX and InBios JE Detect) have shown that they have low sensitivities which ranged between 17% and 57% (Robinson et al., 2010). Furthermore, all these diagnostic methods are confined to qualitative analysis only.

Majority of the JE cases happened in rural areas in Southern and Eastern Asian Countries, where access to diagnostic laboratory facilities is very limited. Mansfield et al. (2017) mentioned JEV infection is difficult to be detected due to a short duration of viremia. Hence, a portable biosensor system that can provide point-of-care, rapid, sensitive and economical diagnostic tool is highly needed for such settings. Undeniably, it has drawn the attention of many researchers in the related field to explore and design different configurations and approaches to preparing biosensors. This is important as many dangerous bacterial infections could be triggered by as low as 10 organisms. Various configurations of biosensors are being explored recently which includes antibody-based system, enzyme-based detection, and DNA-based sensors. The biosensor has proven its high potential in terms of ability to shorten the detection time from 2 to 4 days to less than an hour *via* a simple target extraction method (Wang & Dai, 2015). This is an important discovery in the medicinal field which shows integration in ensuring a fast and easy way to obtain a signal from biological events (Liu & Guo, 2012).